Supramolecular Polymerization from Polypeptide-Grafted Comb Polymers

Jing Wang[1], Hua Lu[2], Ranjan Kamat[1], Sai V. Pingali[3], Volker S. Urban[3],
Jianjun Cheng[2,*], Yao Lin[1,*],
[1] Polymer Program, Institute of Materials Science & Department of Chemistry, University of Connecticut, Storrs, Connecticut 06269, USA; [2] Department of Materials Science and Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA; [3] Center for Structural Molecular Biology, Neutron Scattering Science Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA

Experimental Section

General. All chemicals were purchased from Sigma-Aldrich (St. Louis, Mo) and used as received unless otherwise specified. Anhydrous dimethylformamide (DMF) was dried by columns packed with 4Å molecular sieves and stored in a glove-box. Tetrahydrofuran (THF) and hexane were dried by columns packed with alumina and stored in a glove-box. Grubbs Catalyst[1], *N*-(*N'*-trimethylsilylaminoethylene)-5-norbornene-*endo*-2,3-dicarboximide, *N*-benzyl-5-norbornene-*endo*-2,3-dicarboximide and γ-benzyl-L-glutamate *N*-carboxylanhydride (Glu-NCA) were prepared by following the previously reported procedures.[2-4]

Characterization. NMR spectra were recorded on a Varian UINB 500 MHz, a VXR 500 MHz or a Bruker DRX 500 MHz spectrometer for polymer characterization. Tandem gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (Model 1100, Agilent Technology, Santa Clara, CA), a DAWN HELEOS 18-angle laser light scattering detector (also known as multi-angle laser light scattering (MALLS) detector, Wyatt Technology, Santa Barbara, CA) and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA). The detection wavelength of HELEOS was set at 658 nm. Separations were performed using serially connected size exclusion columns (100Å, 500Å, 10^3Å and 10^4Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA) at 60 °C using DMF containing 0.1 M LiBr as the mobile phase. The MALLS detector was calibrated using pure toluene with no need for external polymer standards and can be used for the determination of the absolute molecular weights of both linear polymer and branched polymers. Laser Desorption/Ionization-Time of Flight mass spectrometry (MALDI-TOF MS) spectra were collected on an Applied Biosystems Voyager-DETM STR system. Circular dichroism (CD) measurements were carried out on a JASCO J-700 spectrometer. A 1mm-pathlength cuvette was used here for CD measurements. The hydrodynamic diameter of the comb polymers was determined by a Malvern Zetasizer Nano S equipped with a 633 nm laser. The comb polymers and the morphologies of the supramolecular structure were characterized with transmission electron microscopy (TEM, Tecnai T12) operating at an accelerating voltage of 120 kV. Samples were deposited on carbon-coated copper grids, blotted by filter paper and subsequently vacuum-dried. The PN-*g*-PLG samples were stained with uranyl acetate (2%) for 15 s. The supramolecular polymers poly(PN-*g*-PLG) were unstained. For the sample prepared from microtome, a voltage of 80 kV was used in the TEM experiments. Field Emission Scanning Electron Microscopy (FESEM) was performed at various magnifications using a JEOL 6335 field-emission scanning electron microscope with an accelerating voltage of 10 kV. The samples were deposited on pre-cleaned glass slides and coated with palladium before imaging. Reflective Fourier-transform infrared spectroscopy (FTIR) was performed on a Nicolet Magna 560 FTIR system equipped with 2x Spectra-Tech IR-Plan microscopes. The samples were deposited on gold-coated glass slides for the FTIR experiments in reflective mode. Laser confocal fluorescence microscopy (LCFM) experiments were performed on an Andor Confocal & TIRF Microscope. The excitation wavelength was chosen at 488 nm and the detection wavelength at 509 nm. Before the experiments, the supramolecular polymers were stained with thioflavin T (ThT) for 15-min at 4 °C. Two-dimensional (2D) WAXD experiments were performed on an Oxford Xcalibur Diffractometer with an ONYX CCD area detector. The X-ray wavelength was Cu Kα 0.1542 nm. One-dimensional (1D) WAXD profiles were obtained by integration from corresponding 2D WAXD images. The *d*-spacing was calibrated using silver behenate with the first-order reflection at a scattering vector $q = 1.076$ nm^{-1}, where $q = (4\pi \sin\theta)/\lambda$ (θ is the half scattering angle). For microtome experiments, the supramolecular polymers assembled from PN_{11}-*g*-PLG_{101} were first casted on silica wafer to obtain a thin film, which was then embedded in epoxy resin. Thin sections of samples with a thickness of around 60 nm were prepared from the rotary microtome and collected on the TEM grids.

Synthesis of PN_x-*g*-PLG_n comb polymers. PN_x-*g*-$PBLG_n$ comb polymers were prepared by following the procedure developed previously[4]. Poly(norbornene-*endo*-2,3-dicarboximide) (PN) bearing *N*-TMS groups was prepared through ROMP in THF or dichlorormethane. After the removal of the solvent under vacuum, the PN was used to initiate the Glu-NCA polymerization in DMF *in situ* without further purification. Comb polymers were usually obtained in 80-95% yield. The MW and MWDs (M_w/M_n) of the comb polymers PN_x-*g*-$PBLG_n$ were determined by GPC and summarized in Table S1.

To deprotect PBLG to obtain PN_x-*g*-PLG_n, PN_x-*g*-$PBLG_n$ (500 mg) was dissolved in TFA (10 mL) in an ice bath and HBr (33 wt% in acetic acid, 2-3 mL) was added to the solution dropwise during a time period of 5 min. The solution was stirred in the ice bath for 2 h. The reaction solution was poured into 40-mL ice-cold dry ether (4 °C). The precipitated polymer was collected by centrifugation, washed by dry ether twice (2 × 30 mL) and dried under vacuum. The crude product was dissolved in NaOH (2 M, 10 mL) and stirred at room temperature overnight. The resultant solution was cooled in an ice bath and its pH was adjusted to 1-2 by adding HCl (1 M). The suspension was dialyzed against DI water for two days and lyophilized to give the PN_x-*g*-PLG_n in almost quantitative yield.

Synthesis of $(PLG_n)_2$ and **$(PLG_n)_3$ polymers.** The synthesis of $(PBLG_n)_2$ and $(PBLG_n)_3$ were performed using previously reported methods[2] using *N*, *N*'-bis(trimethylsilyl) cystamine and 1,3,5-triaminomethyl benezene as initiator, respectively. The deprotection of $(PBLG_n)_2$ and $(PBLG_n)_3$ followed the same protocol described in the synthesis of PN_x-*g*-PLG_n to give $(PLG_n)_2$ and $(PLG_n)_3$ (Scheme S1).

Synthesis of gold nanoparticles (Au NPs). The Au NPs (~28nm) were prepared by the Frens' method[5]. Sodium citrate solution (4.3 mL, 1% (w/v)) was added to a boiling solution of $HAuCl_4$ (290 mL, 0.015% (w/v)). The color of the solution turned wine-red after 3 min, indicating the formation of Au NPs. The solution was boiled for another 10 minutes, cooled to room temperature and the Au NPs were used for the preparation of PLG-grafted gold nanoparticles (PLG-*g*-NPs).

Synthesis of PLG-grafted gold nanoparticles (PLG-*g*-NPs). The PLG-SH ligands were obtained by cleaving the disulfide (S-S) bond in $(PLG_n)_2$ with the addition of tris(2-carboxyethyl)phosphine hydrochloride (TCEP). PLG_n-S-S-PLG_n (0.5 mL, 4 mg/mL) was incubated with TCEP (6 μL, 10 mM) for 1 h with stirring before the addition of Au NPs solution (3mL, 3 nM). The mixture was then incubated overnight. Au NPs were collected by centrifugation, washed by ultrapure water three times to remove the excess PLG-SH, and re-dispersed in water. The pH of the solution was then adjusted to 7 by adding NaOH (1M).

Supplementary Schemes, Tables and Figures

Scheme S1. Synthesis of PLG-containing Y-shaped polymers $(PLG_n)_3$ and block copolymers $(PLG_n)_2$ by controlled ROP-NCAs.

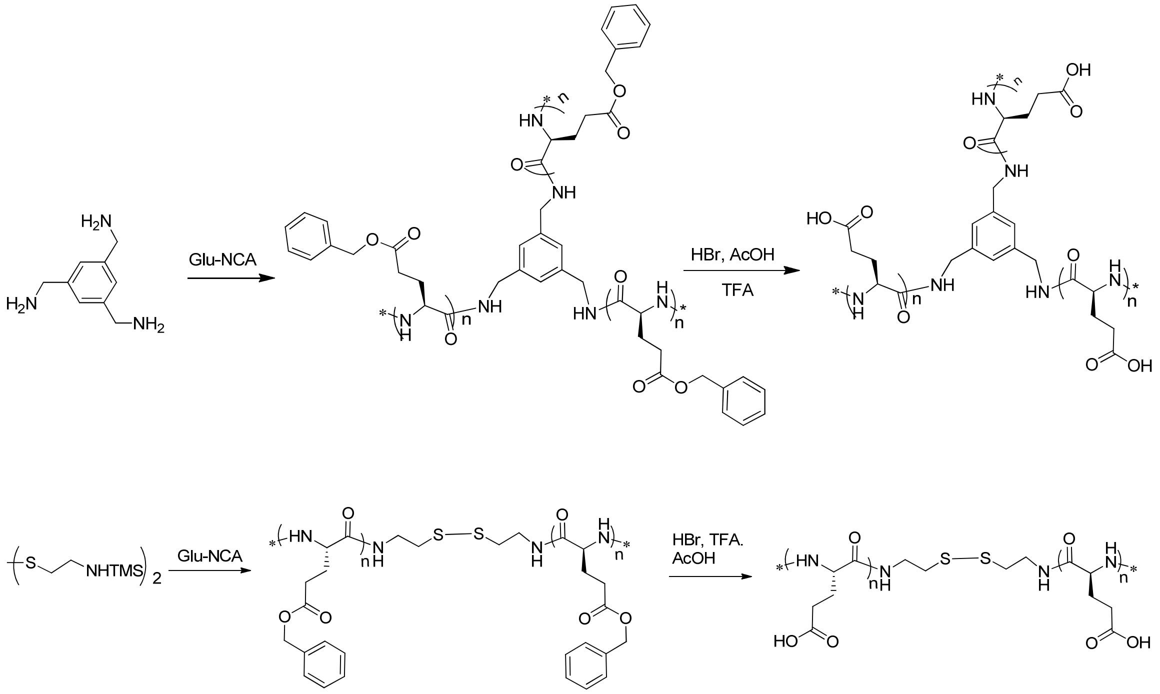

Table S1. Characterizations of the comb-like polymers, Y-shaped polymers and di-block copolymers.

entry	polymer	x(x*) [a]	n(n*)[b]	$M_n(M_n*)^c$ $(\times 10^3$ g/mol)	MWD (M_w/M_n)
1	PN_{11}-*g*-$PBLG_{62}$	11(10)	62 (50)	*151 (111)*	1.28
2	PN_{11}-*g*-$PBLG_{101}$	11(10)	101(100)	*245 (222)*	1.13
3	PN_{18}-*g*-$PBLG_{128}$	18 (20)	128 (100)	*510 (446)*	1.26
4	$(PBLG_{94})_3$		94(100)	*667(711)*	1.12
5	$(PBLG_{97})_2$		97(93)	*459(438)*	1.02
6	$(PBLG_{55})_2$		55(46)	*259(219)*	1.06

[a] x = the obtained degree of polymerization (DP) of ROMP, x* = the expected DP of ROMP; [b] n = the obtained DP of polypeptides, n* = the expected DP of polypeptides; [c] M_n = the obtained M_n; M_n* = the expected M_n.

Table S2. Summary of the tubular supramolecular polymerization from different macromolecular monomers.

Entry	Macromolecular monomer	Supramolecular polymerization	External diameter of the tubular supramolecular structures (nm)
1	PN_{11}-*g*-PLG_{62}	Yes	70±10
2	PN_{11}-*g*-PLG_{101}	Yes	170±20
3	PN_{18}-*g*-PLG_{128}	Yes	420±50
4	$(PLG_{94})_3$	No	-
5	$(PLG_{97})_2$	No	-
6	$(PLG_{55})_2$	No	-

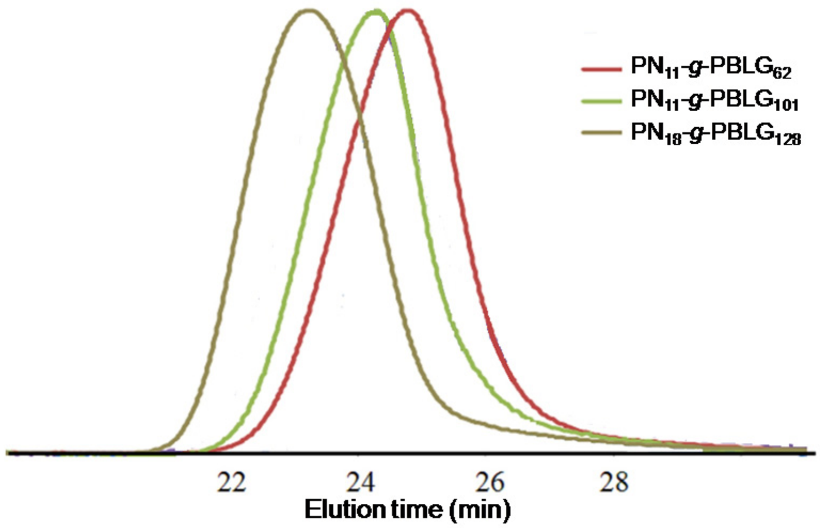

Figure S1. Overlay of the GPC curves (reflective index signals) of PN-*g*-PBLG comb polymers.

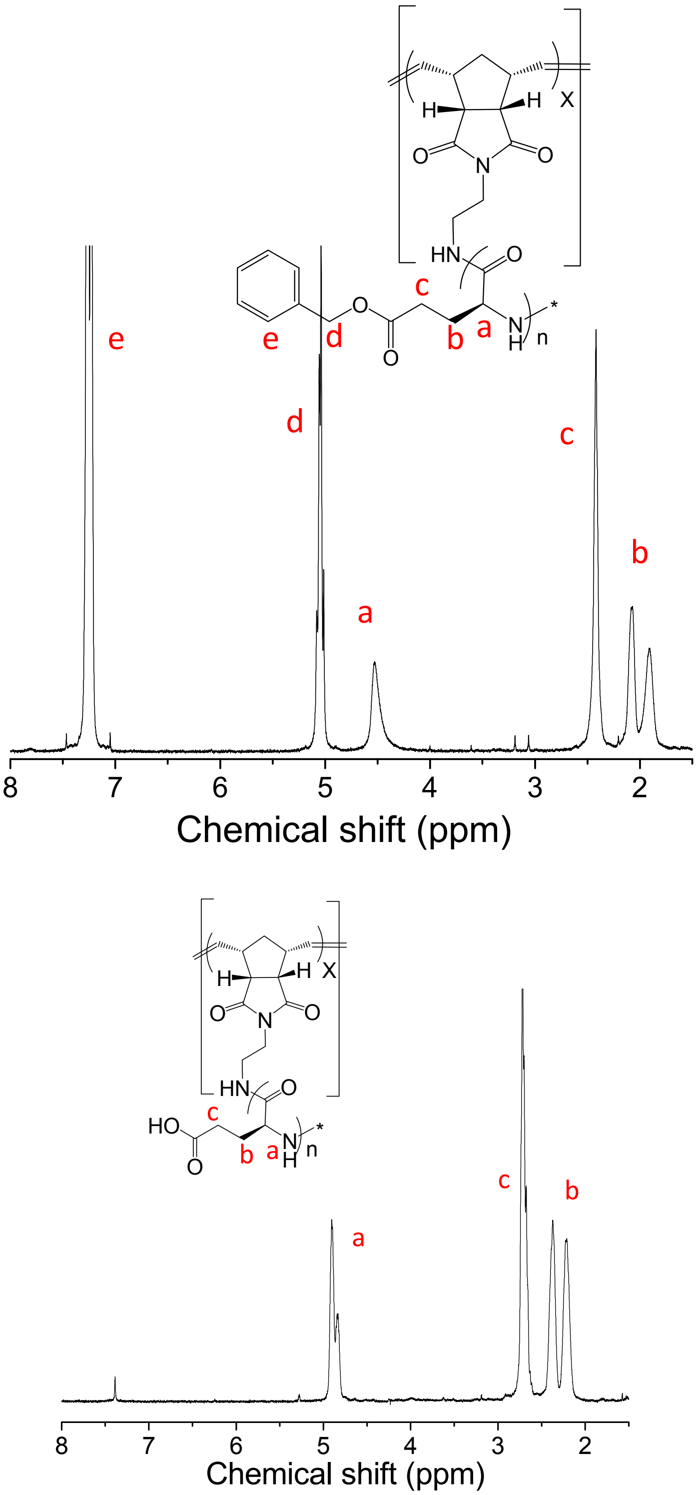

Figure S2. ^{1}H NMR spectra of PN$_{11}$-*g*-PBLG$_{62}$ (upper) and PN$_{11}$-*g*-PLG$_{62}$ (lower) in TFA-*d* (c = 1 mg/mL).

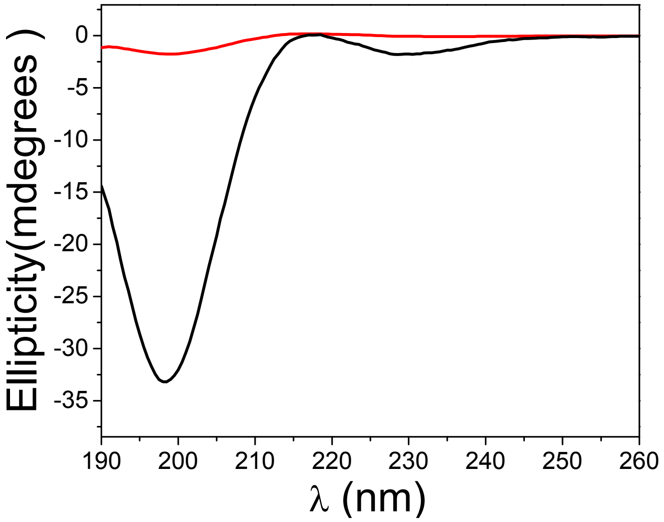

Figure S3. The CD spectra of the freshly-made PN_{11}-*g*-PLG_{101} solution (10 μM, pH 7) (in black) and the solution after 21 days and removal of supramolecular polymers by centrifugation (in red). It showed that the grafted PLGs in dispersed PN_{11}-*g*-PLG_{101} retained their extended coil structures in solution.

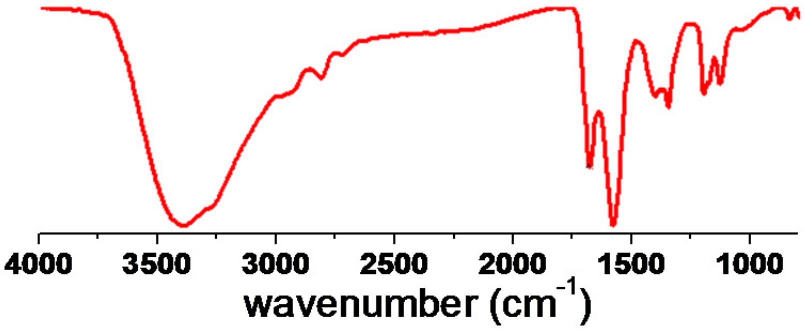

Figure S4. FTIR spectrum of poly(PN_{11}-*g*-PLG_{101}). the FTIR spectrum of supramolecular polymers was in consistent with characteristic anti-parallel β-sheet conformation, based on the assignment of the absorption peaks at 1689 cm^{-1} (amide I), 1632 cm^{-1} (amide I) and 1530 cm^{-1} (amide II). The peak at 1590cm^{-1} was due to the ionized carboxylate groups.[6, 7]

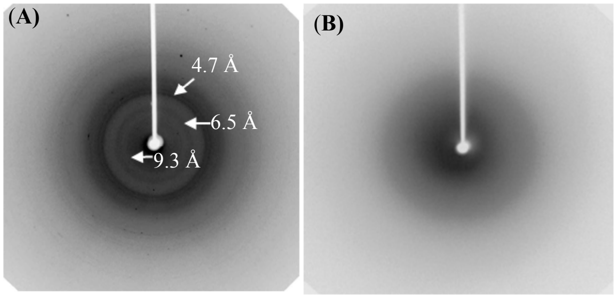

Figure S5. (**A**) Wide angle X-ray scattering (WAXS) of poly(PN_{11}-*g*-PLG_{101}), the supramolecular polymers were collected by centrifugation. (**B**) WAXS of a film of PN_{11}-*g*-PLG_{101}, the sample was prepared by drying a freshly made polymer solution on the surface.

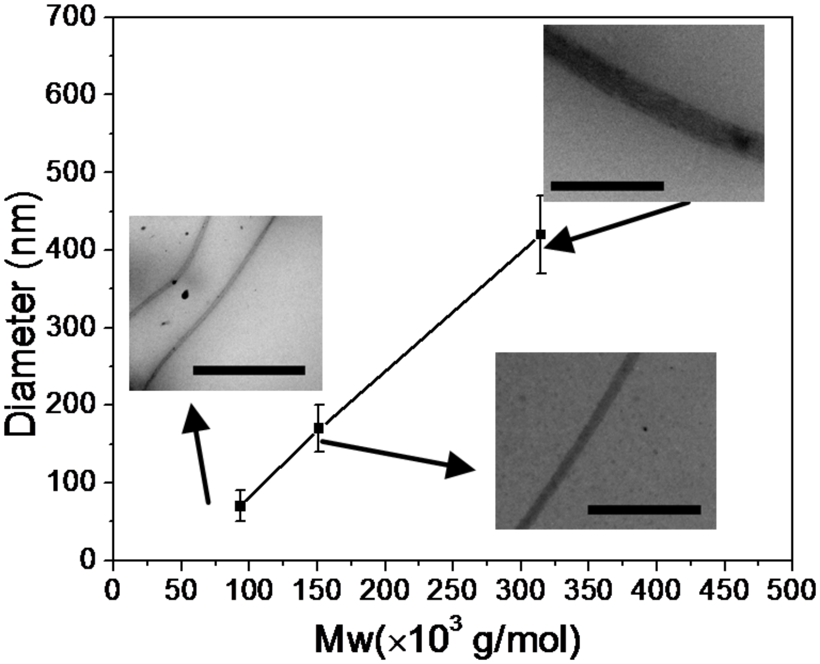

Figure S6. The correlation between the external diameter of the tubular supramolecular polymers and the molecular weight of the macromolecular monomer (PN-*g*-PLG); (inset) TEM images of the tubular supramolecular polymers: poly(PN_{11}-*g*-PLG_{62}), poly(PN_{11}-*g*-PLG_{101}) and poly(PN_{18}-*g*-PLG_{128}). All the scale bars are 2 μm.

References:

1. Choi, T.L. & Grubbs, R.H. Controlled living ring-opening-metathesis polymerization by a fast-initiating ruthenium catalyst. *Angew. Chem.-Int. Edit.* **42**, 1743-1746 (2003).
2. Lu, H. & Cheng, J.J. N-trimethylsilyl amines for controlled ring-opening polymerization of amino acid N-carboxyanhydrides and facile end group functionalization of polypeptides. *J. Am. Chem. Soc.* **130**, 12562-+ (2008).
3. Lu, H. & Cheng, J.J. Hexamethyldisilazane-mediated controlled polymerization of alpha-Amino acid N-carboxyanhydrides. *J. Am. Chem. Soc.* **129**, 14114-14115 (2007).
4. Lu, H., Wang, J., Lin, Y. & Cheng, J.J. One-Pot Synthesis of Brush-Like Polymers via Integrated Ring-Opening Metathesis Polymerization and Polymerization of Amino Acid N-Carboxyanhydrides. *J. Am. Chem. Soc.* **131**, 13582-13583 (2009).
5. Frens, G. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nature-Phys. Sci.* **241**, 20 (1973).
6. Lee, N.H. & Frank, C.W. Surface-initiated vapor polymerization of various α-amino acids. *Langmuir* **19**, 1295-1303 (2003).
7. Shim, J.Y. & Gupta, V.K. Reversible aggregation of gold nanoparticles induced by pH dependent conformational transitions of a self-assembled polypeptide. *J. Colloid Interface Sci.* **316**, 977-983 (2007).